to genomes isolated from tick vectors in order to assess whether there are viral characteristics associated with human infection. Overall, our results highlight the utility of metagenomics NGS to identify and study the molecular epidemiology of viruses that cause CNS infection.

A54 Viral metagenomics: Relative viral enrichment and detection limits in clinical serum and faeces

Dennis Schmitz,[1,2] Jeroen Cremer,[1] Harry Vennema,[1] Annelies Kroneman,[1] and Marion Koopmans[1,2]

[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands and [2]Erasmus Medical Centre (EMC), Rotterdam, The Netherlands

Any etiological agent containing nucleic acids should be identifiable using random next-generation sequencing (NGS) of human clinical materials. Unlike PCR based and baiting strategies, random NGS metagenomics is able to identify unknown and genetically drifted agents without relying on prior knowledge. This makes it suitable for the identification of RNA-viruses, which naturally drift due to their error-prone RNA-dependent RNA polymerase. However, NGS applied to virome investigation (viral metagenomics) presents biological and technical barriers. Viruses often cannot be cultured or isolated, therefore, low viral load samples are common. Furthermore, during NGS, all nucleic acid molecules compete for limited sequencing capacity (whether viral or non-viral) and the costs of NGS increases proportional with required sequencing depth. Therefore, to efficiently sequence low viral load samples, a protocol has to be developed that enriches viruses/viral nucleic acid. We first focused on single stranded RNA-viruses in serum and faeces. Different stages of the protocol were tested in the process from RNA-virus positive sample to dsDNA input for NGS: centrifugation, filtration, endonuclease treatment, RNA extraction, reverse transcription, second strand synthesis and library preparation. Different combinations of these methods were applied to human faeces and serum and assessed using qPCR. Subsequently, the optimal method was applied to Chikungunya virus positive serum and norovirus positive faeces ranging from Ct eight and eleven up to Ct 35 and 30, respectively. Lastly, these samples were sequenced using an Illumina MiSeq (PE300, $\sim 10^6$ reads/sample) and analyzed to determine detection limits. Our method reliably generates full ($>$95 per cent) viral genomes up to Ct 26 in both serum and faeces, while allowing identification of viral agent up to Ct 30. Viral metagenomics proved its merit by also identifying sapovirus, coxsackievirus, parechovirus, and picobirnavirus in faeces. The coxsackievirus and parechovirus were confirmed using qPCR with a Ct of 28 and 29.18, respectively. The identified sapovirus could not be confirmed using our diagnostic qPCR, although NGS data coverage indicated a high viral load. Further analysis of this sapovirus showed many mutations in the qPCR primer binding site, explaining the negative result in our diagnostic assay. These results emphasize the power and promise of viral metagenomics.

A55 Foot-and-mouth disease virus undergoes abundant viral genomic changes at distinct stages of infection of cattle

I. Fish,[1,2] C. Stenfeldt,[1,2,3] S. J. Pauszek,[1] B. P. Brito,[1,2] E. J. Hartwig,[1] G. Smoliga,[1] L. L. Rodriguez,[1] and J. Arzt[1]

[1]Foreign Animal Disease Research Unit, United States Department of Agriculture, Plum Island Animal Disease Center, Agricultural Research Service, Greenport, NY, USA, [2]Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, TN, USA and [3]Department of Veterinary Population Medicine, University of Minnesota, Twin Cities, MN, USA

The rapid evolution of pathogenic RNA viruses presents a major challenge for scientists and others fighting to control transmission, predict and prevent future epidemics. Foot-and-mouth disease virus (FMDV), the picornavirus responsible for the eponymous disease, is one of the costliest livestock pathogens across much of the globe. Understanding how the virus changes over time both within hosts and through chains of transmission is of central importance for vaccine development, vaccination and quarantine strategies and international trade regulations. Cloven-hoofed animals including swine, cattle, and other domesticated and wild bovids are susceptible to the disease. Importantly, cattle and buffalo can be long-term carriers of the virus with the role of these animals in transmission being an active subject of research. Recent publications examining the full-length FMDV genome have begun to explain the complexities of the quasispecies and its behavior through transmission events and within hosts. Several of our lab's recent publications have addressed the question of which factors are responsible for inducing the carrier state. Our current endeavors build upon these concepts with detailed genomic study of FMDV in experimentally infected cattle through the acute and persistent phases of infection. Beginning with a heterogeneous inoculum mirroring the diversity that might be seen in an intensive farm outbreak, we have followed the progression of consensus genomes in twelve steers through different stages of disease including incubation, clinical disease, and the post-acute carrier state. In this study, we have documented convergent novel mutations at the canonical host cell entry RGD motif. We also characterized divergent minority genomes that, through powerful selective sweeps, became dominant at distinct points of infection. This study included both vaccinated and unvaccinated animals, with protection correlating with different patterns of viral evolution, notably at major antigenic sites. This is the first study to evaluate the full consensus genome of FMDV at distinct stages of infection, thus revealing significant micro-evolutionary events that can be of substantial benefit to disease control strategies and epidemiological modeling. The next stage of this work, supported by preliminary NGS data, will incorporate quasispecies-level analysis, elucidating the dynamic selective and population pressures during viral infection.

A56 Evolutionary analyses of foot-and-mouth disease virus in Southeast Asia using whole-genome sequences

Barbara Brito,[1,2] Steven J. Pauszek,[1] Ethan J. Hartwig,[1] George R. Smoliga,[1] Le T. Vu,[3] Pham P. Vu,[3] Carolina Stenfeldt,[1,2] Luis L. Rodriguez,[1] Donald P. King,[4] Nick J. Knowles,[4] Kasia Bachanek-Bankowska,[4] Ngo T. Long,[3] H. Dung,[5] and Jonathan Arzt[1]

[1]Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, ARS, USDA, NY, USA, [2]Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, TN, USA, [3]Department of Animal Health, Ministry of Agriculture and Rural Development, Regional Animal Health Office No 6, Ho Chi Minh City, Vietnam and [4]The Pirbright Institute, Pirbright, UK

Foot-and-mouth disease (FMD) is one of the most important diseases of livestock worldwide. The causative agent, FMD virus (FMDV) is an aphthovirus from the Picornaviridae. The FMDV ORF is translated as a single polyprotein that codes for four structural proteins and eight non-structural proteins. Molecular epidemiology and evolution of FMDV have been traditionally studied using the sequence coding for VP1 (639 nt), the capsid protein containing most relevant antigenic domains. Although full-genome sequencing of this virus is not used as a routine diagnostic or surveillance tool, the availability of full-genome sequences in public repositories has increased over recent years.